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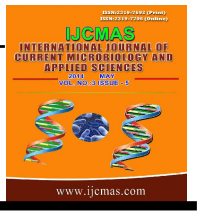

Original Research Article

Tannery effluents de-colorization efficiency of bacterial isolates from River Yamuna and industrial effluents

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A B S T R A C T

Treatment methodologies of dye containing wastewater fall into three types: physical, chemical and biological. Biological methods have been proved to be superior over various physicochemical methods because of lower cost. Biological methods are safe for ecology also. Therefore, microorganism isolated from the site of pollution will be tool of interest for treatment of the effluent. We isolated 11 bacterial cultures from river Yamuna water and tannery industrial effluent (5 & 6 cultures respectively). Of these 7 cultures were classified as Gram negative cocci, 3 cultures as Gram negative bacilli and remaining one as Gram positive bacilli. All these isolates were screened for Acid Black dye de-colorization efficiency using bacterial culture in dye supplemented solid and liquid culture media. Finally, spectrophotometric analysis carried out to evaluate percent de-colorization of Acid Black dye and thin layer chromatography analysis was carried out to study dye de-colorization mechanism. All the cultures showed dye de-colorization property against tannery dye Acid Black (7.91% to 95.77%). The most promising cultures (encoded as S.5.2) from Tannery effluents showed more than 90% de-colorization while cultures (encoded as S.2.1 & S.5.1) from Yamuna water effluents showed >50% de-colorization. TLC analysis confirmed the dye degradation property of cultures S.5.2, as there were no dye specific spots in comparison to dye sample. The Yamuna water and effluent both have efficient dye de-colorization bacteria. TLC and bacterial pellet had showed decolouriszation of the dye by adsorption and degradation. Strain S.5.2 can be proposed for further development of tannery waste water clarification system.

Keywords

Tannery effluents, Biodegradation, Thin layer chromatography, pure culture, spectro-photometric analysis

Introduction

Potable water is of vital importance for human existence. Water is useful for domestic, industrial and agricultural purposes. The past decade has seen remarkable impact of man on the environment due to unprecedented increase in population and rapid rate of urbanization as well as the intensification of the use of fragile and marginal ecosystems. Indian tannery Industry is one of the leading tannery industries in the world. It is also one of the highly polluting industries in the State. Tannery effluents are having potential to create pollution of

water and air. Taking into account the volume and composition of effluent, the tannery wastewater is rated as the most polluting among all in the industrial sectors [1-2]. In general, the wastewater from a typical tannery industry is characterized by high values of BOD, COD, color and pH [3-4]. It induces persistent color coupled with organic load leading to disruption of the total ecological/symbiotic balance of the receiving water stream [5]. Incomplete use and the washing operations give the tannery wastewater a considerable amount of dyes [6]. The dyes are xenobiotic in nature and in some cases are mutagenic and carcinogenic [7-8]. Allergic effects of these dyes have also been reported by several workers [9-10]. Without adequate treatment, the dyes can remain in the environment for a long period of time. Traditionally, both physical and chemical methods such as coagulation, ozonation [11], precipitation, adsorption by activated charcoal, ultrafiltration, nanofiltration [12], electrochemical oxidation, electrocoagulation [13-14] etc were used in the treatment of the tannery industrial effluents [2]. But most of these methods are not accepted by the industries due to their high cost, low efficiency and inapplicability to a wide variety of dyes [15-20].

However, in situ degradation of the effluent is a novel method under the biodegradation process. In this method, the microorganisms isolated from the site of pollution can be used for the treatment of the effluent [5, 21]. So, present study was designed to isolate efficient dye de-colorization bacterial strains from the Yamuna water as well as tannery effluents. Since the bacterial isolates were originated from the dye contaminated tannery wastewater of local industry, so they can easily adapt to the prevailing local environment. Therefore, such bacteria can be used to develop an effective biological treatment system for the wastewaters contaminated with dyes.

Materials and Methods

Isolation and purification of bacteria from Yamuna water and tannery effluents

The bacterial cultures were isolated from Yamuna water and tannery effluent samples by serial dilution method and purified by repeated streaking. Each of the pure bacterial culture was maintained in the slants as a pure or stock culture. All the cultures were coded as given in table 1 and 2.

Bacteria were isolated by serial dilution which is done as in the following method described [22-24]. One set of ten tubes containing 4.5ml of normal saline buffer (0.8% NaCl) were prepared, 0.5ml of Yamuna water was transferred to tube one and the content was mixed, then tube two was inoculated with 0.5ml of tube one. Using this method, we prepared dilution series with 1:10 steps. Next, 100µl of each dilution was spread on nutrient agar plates (HiMedia, India) and then were incubated overnight at 37°C. Different types of obtained colonies were streaked out on fresh plates and transferred at least three times for purification. Bacterial isolate were compared by colony morphology and colour [23]. Finally five bacterial isolate were purified and inoculated to nutrient broth separately and put into a shaker incubator at 120 rpm and 37°C. Similar set of experiments were applied to isolate bacteria from tannery effluents six different isolates were obtained from tannery effluents.

Characterization of bacterial cultures

The pure cultures were characterized on the basis of colony morphology (color, shape, elevation and optical characteristics) and Gram's staining reaction.

Screening of dye de-colorization bacterial cultures

Method of screening bacterial capability to decolourize the Acid Black dye was adapted with modification from Yang et al [25].

De-colorization in solid culture media

Nutrient agar supplemented with Acid Black dye at the concentration 100 mg/L was poured into Petri-plates & inoculated with fresh bacterial culture obtained after 24h by streaking and incubated at 37°C for 14 days. Non-inoculated Petri-plates were also incubated and treated as negative control.

De-colorization in liquid culture media

20 mL of nutrient broth supplemented with Acid black dye at the concentration 100 mg/L was poured into culture bottles and inoculated with fresh bacterial culture obtained after 24h (5% inoculum). Non-inoculated culture bottles were also incubated and treated as negative control. The inoculated and control bottles were incubated at 37°C for 14 days and then observed for disappearance/color change. After completion of the incubation period 10 mL of sample was withdrawn from each of the culture as well as control tube and centrifuged at 10,000 rpm for 10 min. The supernatant were utilized for Spectrophotometric analysis and thin layer chromatography (TLC).

a) Spectrophotometric analysis

Absorbance of the supernatant obtained from the culture as well as the control tubes were recorded at three dye specific wavelengths: 311nm, 391nm and 597nm (Visible range). Sterilized nutrient broth was used as blank. Experimentations in triplicate were considered for the calculations and the results, are presented as mean values along with standard deviation values. Absorbance values of samples and control at 391nm were utilized to calculate percent de-colorization (a measure of de-colorization efficiency) for the bacterial cultures according to the following formula:

$$\textbf{Percent de-colorization} = \frac{(Ao - A) \times 100}{Ao}$$

Here,

Ao = Absorbance of control at the λmax (nm) of the dye (391nm).

A = Absorbance of culture supernatant after incubation period (14 days) at the λmax (nm) of the dye (391nm).

b) TLC analysis: Silica gel plate was prepared using slurry of silica gel (50g) plus Gypsum (7.5 g) in 100 mL of distilled water poured gently on the TLC plate with the help of spreader. After this TLC plate was air dried for 10 min and then activated in hot air oven at 100-110^{o}C temp for 30 min. To load the dye and decolourized sample at a uniform level, a faint line was marked gently with a pencil, 1 cm from the bottom of the TLC plate. Spots of the dye sample and supernatant of de-colorization culture (S.5.2) was applied using capillary tubes on the line and allowed to dry for a few minutes. This step was repeated 4 times.

Then TLC solvent (60 ml ammonium hydroxide + 360 mL 1-propanol) was poured in the TLC chamber and covered with glass plate and after that TLC plate was placed in the chamber with the pencil line containing the spots of dye dipping in the solvent and again covered with glass plate. The solvent was allowed to move up the solid support by capillary action until it gets near the top of the support. The TLC plate was taken out and the position of the solvent (liquid) front marked with a pencil and then allowed to air dry. Conc. HCl was sprayed on TLC plate and left for drying in hot air oven at 110^{o}C for 8 min. Each spot appearing on the TLC plate was marked and distances to the center of the dye spots and to the solvent front was measured. The relative mobility (Rm) value for each dye spot was also calculated according to the following formula:

$$\text{Rm value} = \frac{\text{Distance substance traveled}}{\text{Distance mobile phase (solvent) traveled}}$$

Results and Discussion

Isolation and purification of bacteria from Yamuna water & Tannery effluents

From all samples of Yamuna water five bacterial cultures were isolated whereas from tannery effluents six cultures could be obtained. In total 11 bacterial cultures could be purified and their respective names are given in table-1.

Characterization of bacterial cultures

On the basis of colony morphology it was observed that the cultures from Yamuna water were either white or pale yellow in color, whereas cultures from tannery effluent were orange, yellow, white or colorless. On the basis of the remaining parameters (shape, elevation and optical characteristics) the cultures were showing variable morphology.

All cultures from Yamuna water were Gram negative (table-2, Figure1), in which five were cocci in shape while one was bacilli. Majority of the cultures from tannery effluent were also Gram negative in which five were cocci while only one culture was Gram positive bacilli. Individual characters of all the cultures have been given in table 2.

Screening of dye de-colorization bacterial cultures

De-colorization in solid culture media

All the cultures were growing profusely on the dye supplemented solid media (NA). On the visual analysis partial de-colorization of the dye could be observed in comparison to control (figure – 2; A and B).

De-colorization in liquid culture media

All the cultures showed good growth in the dye supplemented liquid culture media (Nutrient Broth). On visual analysis of the culture tubes, 4 cultures (S.1.1, S.4.1, S.4.2, S.5.3) were not showed any color change in comparison to control and remaining 7 culture tubes (S.2.1, S.3.1, S.3.2,S.3.3, S.5.1, S.5.2, S.6.1) showed disappearance of black colour by varying degree (figure – 3; A- B). After centrifugation when the pellets of the bacterial cultures were observed visually, it was found that the pellet of all the bacterial cultures was black in color suggesting de-colorization mainly by adsorption.

a)Spectrophotometric analysis

Spectrophotometric analysis showed that all the cultures had absorbance values lower than the control, at the dye specific wavelength i.e.; 311nm, 391nm and 597nm (table -3; figure-4).

All the 11 cultures showed de-colorization, although to variable extent 7.91% to 95.77%. The culture S.1.1, S.4.1, S.4.2, S.5.3 were not showing any color change in comparison to control (figure-4) but on the basis of spectrophotometric analysis they were showing slight de-colorization (7.91% to 18.41%) (table-4). The cultures showing color decolourization in comparison to control included culture no.S.2.1, S.3.1, S.3.2, S.3.3, S.5.1, S.5.2, and S.6.1. These cultures were showing de-colorization in the range from 20.84% to 95.77% (table-4). One culture S.5.2 was showing more than 90% de-colorization at 391nm and were considered as the most promising cultures. Cultures S.2.1, S.4.2 & S.5.1 could be also considered as good cultures since they were showing more than 50% de-colorization at 391nm.

b) TLC analysis

After TLC analysis the dye sample showed 3 spots i.e. spot 1- yellowish black having Rm value 0.258, spot 2- light purple having Rm value-0.516 and spot 3- pink having 0.761. (figure -5 A & B)
In case of sample S.5.2 no spot was obtained after development. Observations suggest that the acid black dye might have been degraded by the respective bacterial cultures during the incubation period.

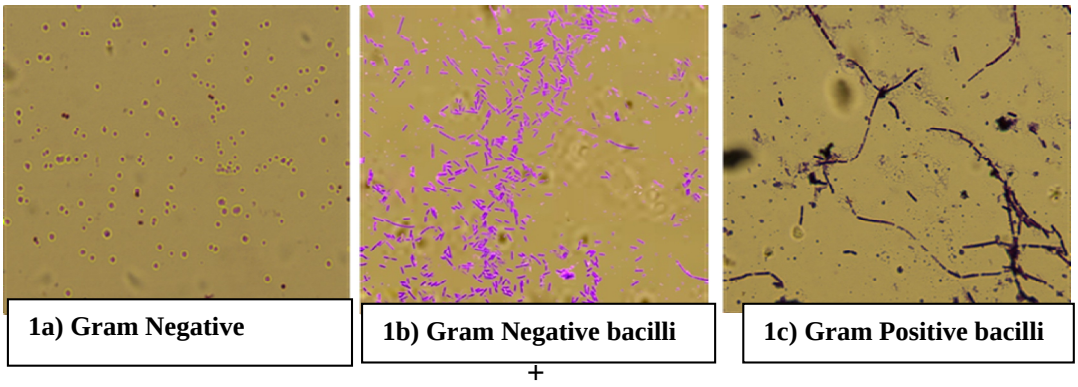

Figure –1 Gram staining results of bacteria from (1a) & (1b) Yamuna water and (1c) from tannery effluents.

(A) **(B)**

Figure.2 Partial Dye decolourisation by bacteria in Petri-plate method. A) Culture plate showing slight partial de-colorization of the dye B) Negative control plate

(A) **(B)**

Figure.3 Dye decolourisation by bacteria in liquid cultures method A) culture (S.5.2) showing dye decolorization B) Negative control

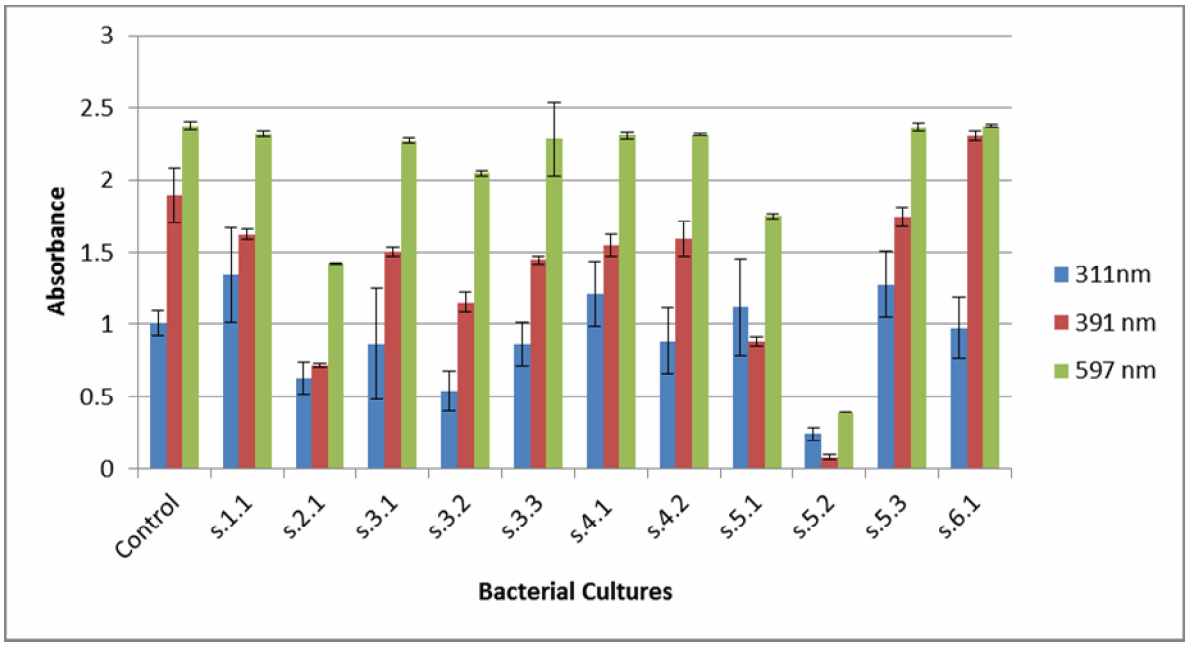

Figure.4 Absorbance values of control and culture supernatant at dye specific wavelengths (bars representing SD values).

(A) (B)

Figure-5: After TLC analysis the A). Sample S.5.2 treated dyes does not show any spot while (B). Untreated dye sample showed 3 spots i.e. yellowish black, purple and pink respectively.

Table.1 List of bacterial cultures isolated from Yamuna water & Tannery effluent samples

Sample no.	Type of cultures isolated	Name of isolated cultures
1.(Yamuna)	1	S.1.1
2.(Yamuna)	1	S.2.1
3.(Yamuna)	3	S.3.1 S.3.2 S.3.3
4. (Tannery)	2	S.4.1 S.4.2
5. (Tannery)	3	S.5.1 S.5.2 S.5.3
6. (Tannery)	1	S.6.1

Table.2 Colony morphology and Gram staining of isolated bacterial cultures from Yamuna water and tannery effluent water

Culture Name	Gram's stain	Shape (Bacteria)	Color	Shape (Colony)	Elevation	Optical Characteristics
S.1.1	Gram Negative	cocci	Yellow	Spherical	Convex	Opaque
S.2.1	Gram Negative	cocci	Orange	Spherical	Convex	Opaque
S.3.1	Gram Negative	bacilli	White	Spherical	flat	Transparent
S.3.2	Gram Negative	cocci	Pale yellow	Spherical	Convex	Opaque
S.3.3	Gram Negative	cocci	White	Spherical	Convex	Transparent
S.4.1	Gram Negative	cocci	White	Spherical	flat	Opaque
S.4.2	Gram Negative	cocci	Colorless	Spherical	Convex	Transparent
S.5.1	Gram Negative	cocci	White	Irregular	flat	Translucent
S.5.2	Gram Negative	cocci	White	Irregular	flat	Opaque
S.5.3	Gram Negative	cocci	white	Irregular	flat	Opaque
S.6.1	Gram Positive	bacilli	Orange	Spherical	Convex	Opaque

Table.3 Absorbance values of control and culture supernatant at dye specific wavelengths

S.No.	Culture name	Absorbance(Mean ± SD)		
		311nm	391nm	597nm
1	**Control**	1.004±0.084	1.895±0.19	2.375±0.025
2	**S.1.1(**Y)	1.342±0.329	1.623±0.038	2.319±0.023
3	**S.2.1(**Y)	0.626±0.111	0.713±0.013	1.423±0.0043
4	**S.3.1(**Y)	0.864±0.386	1.504±0.028	2.269±0.015
5	**S.3.2(**Y)	0.536±0.138	1.152±0.071	2.05±0.014
6	**S.3.3(**Y)	0.863±0.153	1.447±0.0226	2.284±0.251
7	**S.4.1(**T)	1.21±0.225	1.546±0.075	2.306±0.03
8	**S.4.2(**T)	0.886±0.232	1.591±0.119	2.313±0.0081
9	**S.5.1(**T)	1.12±0.335	0.883±0.028	1.749±0.017
10	**S.5.2(**T)	0.245±0.044	0.08233±0.0149	0.39±0.0004
11	**S.5.3(**T)	1.277±0.232	1.745±0.064	2.367±0.249
12	**S.6.1(**T)	0.975±0.211	2.305±0.037	2.375±0.0109

Table.4 Percent decolourization of cultures at 391nm

S.No.	Culture No.	% decolourization
1	S.1.1(Y)	14.35
2	S.2.1(Y)	62
3	S.3.1(Y)	20.84
4	S.3.2(Y)	39.31
5	S.3.3(Y)	23.48
6	S.4.1(T)	18.41
7	S.4.2(T)	16.04
8	S.5.1(T)	54
9	S.5.2(T)	95.77
10	S.5.3(T)	7.91
11	S.6.1(T)	21.63

***Y = Yamuna *T= Tannery**

The present study was carried out to isolate bacterial cultures from Yamuna water & tannery effluents, having capability to decolourize tannery dyes. In totality 11 microbial cultures were purified, which can be termed as native micro flora of the Yamuna water and tannery effluent samples as no growth was obtained on plates of nutrient agar, and this ruled out any possibility of the laboratory contamination.

Presence of these cultures in the samples suggests that they are adapted to their polluted environment. The 11 bacterial cultures obtained from Yamuna water and tannery effluents were characterized on the basis of colony characteristics and Gram's reaction. When the isolates were compared on this basis of colony characteristics it was found that some isolates from Yamuna were showing quite similar colony characteristics, but most of the isolates from tannery effluents were different on this criteria. Result indicates that there was more diversity in the bacterial micro flora of tannery effluents. Also it was observed that isolates from the Yamuna and tannery effluents were showing quite different colony characteristics, which means that isolates obtained from Yamuna water were different from the isolates of tannery effluents.

Yamuna water contributed Gram negative cocci and Gram negative bacilli while tannery effluent contributed Gram negative cocci, and Gram positive bacilli. It might be concluded that Gram negative cocci is more adapted to grow in the polluted environment. Also it can be concluded that the Yamuna and tannery effluents were quite distinct in terms of bacterial groups inhabiting there, which is parallel to the results of colony characteristics.

These isolates were further screened for their dye de-colorization capabilities. When the bacterial cultures were subjected to the dye de-colorization experiment in solid culture medium against the Acid Black dye, it was found that all the 11 isolates were de-colorization the dye only partially on the solid media. This might be due to the unavailability of the dye molecules for the bacterial metabolism which were bound in the solid medium. These findings were similar in effect of media as demonstrated by Barraga´n et al [26].

In case of liquid culture medium it was found that all the cultures were de-colorization the dye Acid black at least to some extent. De-colorization of dye solution may take place in two ways, either adsorption on the microbial biomass or biodegradation of the dye molecules by the bacterial cells [27]. Dye adsorption may be evident from the inspection of the bacterial growth, those adsorbing the dye was deeply colored (similar in color to the adsorbed dye), while those degrading the dye will remain colorless [28-30]. In this experiment all of the isolate were found to be colored with dye Acid Black after completion of de-colorization experiment, indicating that these isolates were de-colorization the dye mainly by adsorption but the biodegradation also cannot be ruled out.

After spectrophotometric analysis it was observed that the most promising culture (S.5.2) showing more than 90% de-colorization were from tannery effluent and this might be due to their adaptation for dye contaminated environment. The other two efficient cultures (S.2.1 and S.5.1) showing >50% de-colorization were from Yamuna and tannery effluent, respectively, suggesting that they developed adaptive capability due to their growth in dye contaminated environment. The dye degradation by S.5.2 was further supported by TLC analysis. On TLC analysis for cultures S.5.2, it was clearly indicated that this culture decolorized the dye by adsorption as well as biodegradation since no dye specific spot could be obtained after incubation in comparison to dye sample (figure 5). So present study suggested the presence of bacteria in Yamuna water and tannery effluents which are able to remove Acid Black dye from waste water by adsorption and biodegradation methods.

The tannery effluent and Yamuna water harbour bacteria which have capability to decolourize tannery dye. Some of the native bacteria isolated from Yamuna water (S.2.1) & tannery effluent (S.5.2 & S.5.1) respectively were having the capability to decolorize the tannery dye Acid Black efficiently. These bacteria encoded as S.5.2 will be an asset to a water treatment system.

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